Nucleic Acids

- **Nucleic acids** represent the fourth major class of biomolecules (other major classes of biomolecules are proteins, carbohydrates, fats)
- **Genome** - the genetic information of an organism

Information specifying protein structure

- **Transcription** - copying of the DNA sequence information into RNA
- **Translation** - Information in RNA molecules is translated during polypeptide chain synthesis

- Information flow:
- **DNA** $\Rightarrow$ **RNA** $\Rightarrow$ **PROTEIN**

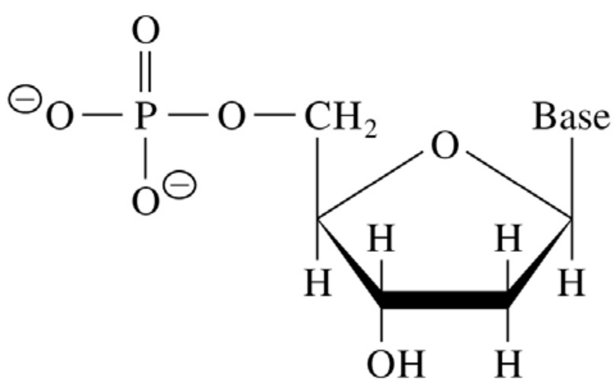
19.1 Nucleotides Are the Building Blocks of Nucleic Acids

• Nucleic acids are polynucleotides

• **Nucleotides** have three components:
  (1) A five-carbon sugar
  (2) A weakly basic nitrogen base
  (3) Phosphate

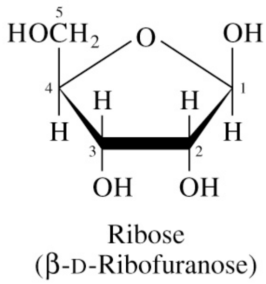
• Nucleotides are phosphate esters of **nucleosides**

Fig 19.1 Chemical structure of a nucleotide

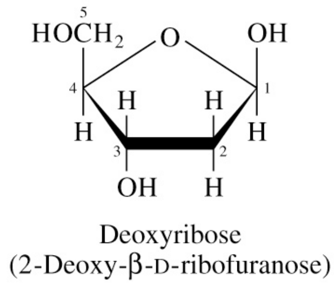


A. Ribose and Deoxyribose

Figure 19.2



In RNA



In DNA

B. Purines and Pyrimidines

Figure 19.3

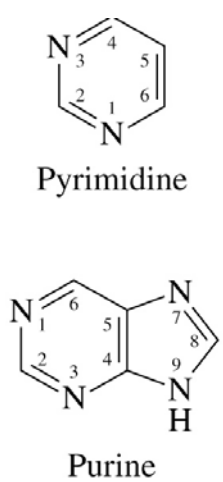


Fig 19.4 Major pyrimidines and purines

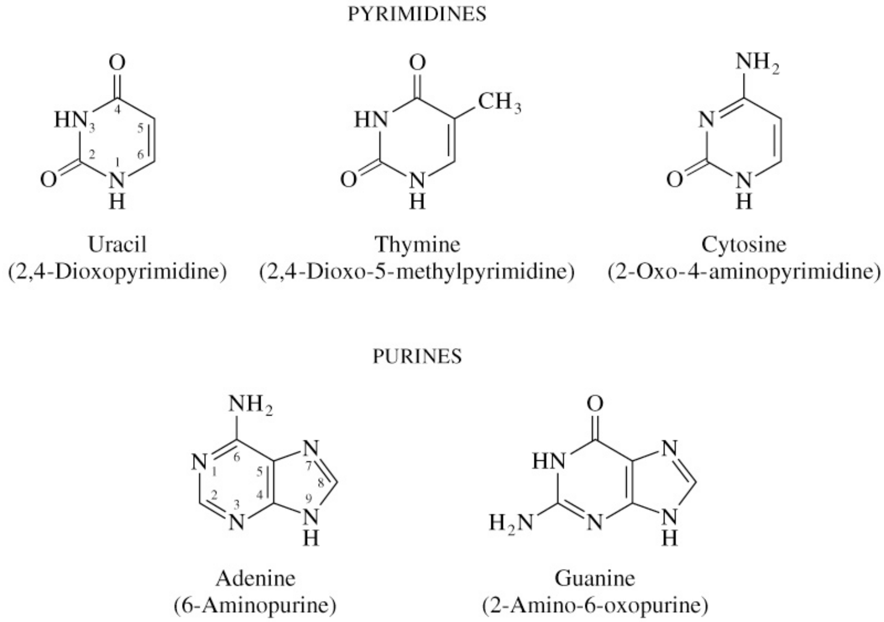


Fig 19.5 Tautomers of adenine, cytosine, guanine, thymine and uracil

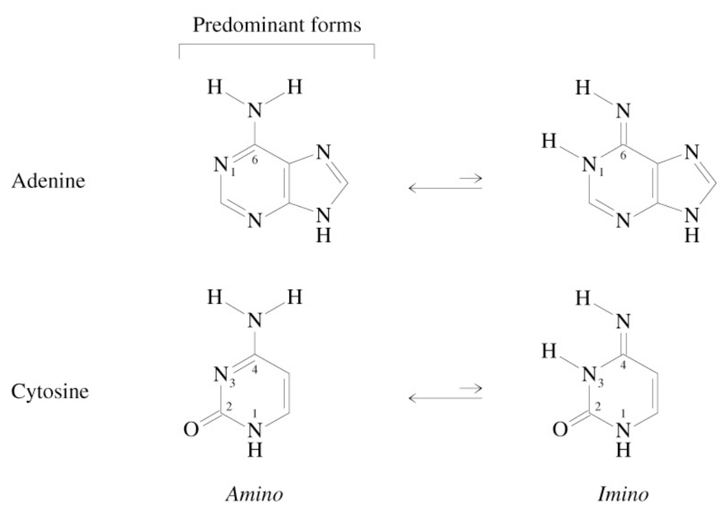


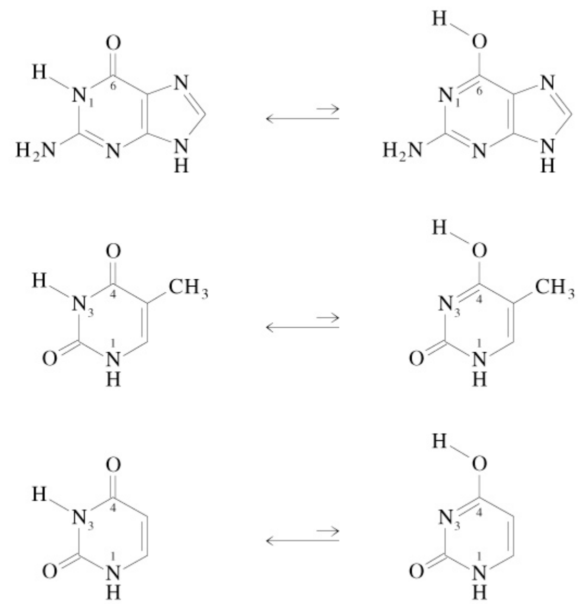
Fig 19.5 (cont)

Keto form predominates

Guanine

Thymine

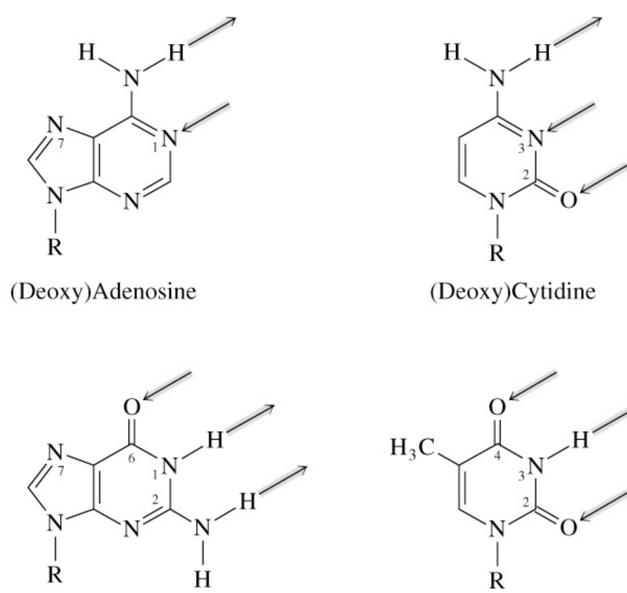
Uracil



Lactam *Lactim*

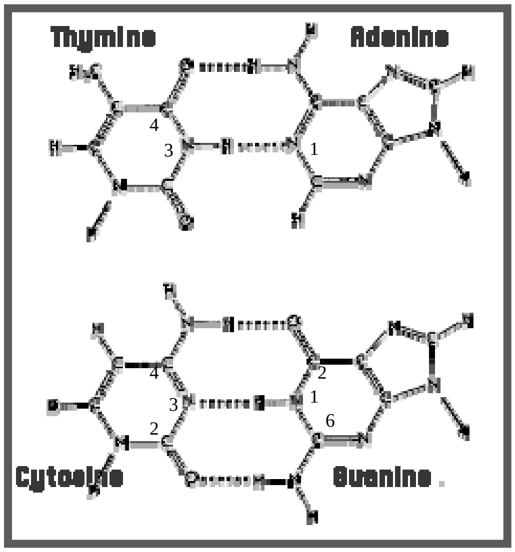
Fig 19.6

- Hydrogen bond sites in nucleic acids



(Deoxy)Adenosine (Deoxy)Cytidine

(Deoxy)Guanosine (Deoxy)Thymidine



C. Nucleosides

Figure 19.7 (a) Nucleoside structures

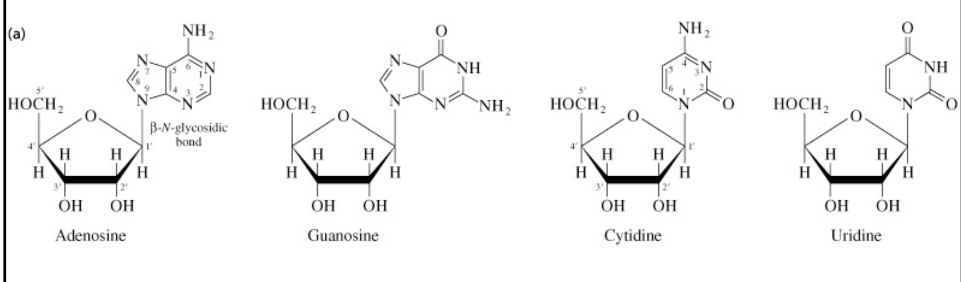
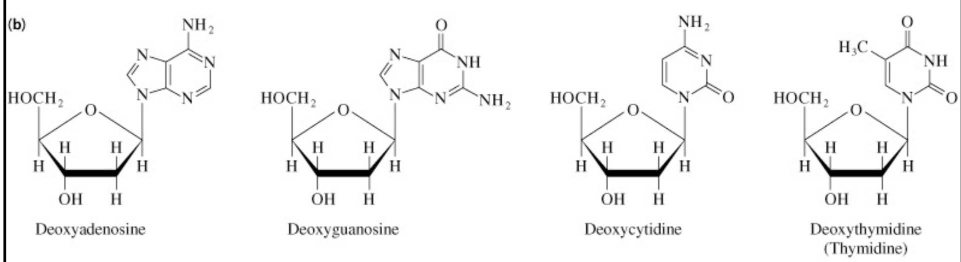


Figure 19.7 (b)

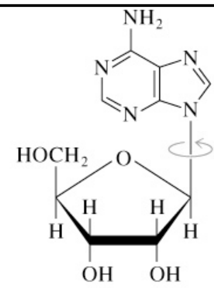


D. Nucleotides

- Nucleotides are phosphorylated derivatives of nucleosides
- Ribonucleosides contain three potential hydroxyl groups (2’, 3’ and 5’)
- Deoxyribonucleosides can be phosphorylated at the 3’ and 5’ positions
- A nucleotide is assumed to be <u>5’-phosphate</u> unless specified otherwise

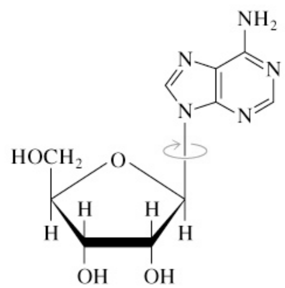
Fig 19.8

- *Syn* and *anti* conformations of adenosine



syn Adenosine

Anti is usually more stable



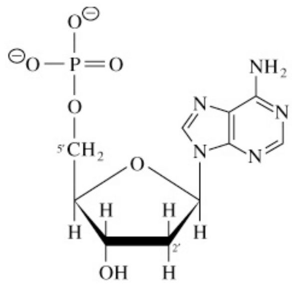
anti Adenosine

TABLE 19.1 Nomenclature of bases, nucleosides, and nucleotides

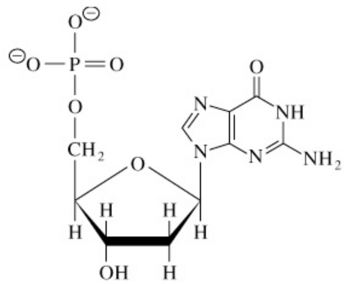
Base	Ribonucleoside	Ribonucleotide (5′-monophosphate)
Adenine (A)	Adenosine	Adenosine 5′-monophosphate (AMP); adenylate[a]
Guanine (G)	Guanosine	Guanosine 5′-monophosphate (GMP); guanylate[a]
Cytosine (C)	Cytidine	Cytidine 5′-monophosphate (CMP); cytidylate[a]
Uracil (U)	Uridine	Uridine 5′-monophosphate (UMP); uridylate[a]
Base	**Deoxyribonucleoside**	**Deoxyribonucleotide (5′-monophosphate)**
Adenine (A)	Deoxyadenosine	Deoxyadenosine 5′-monophosphate (dAMP); deoxyadenylate[a]
Guanine (G)	Deoxyguanosine	Deoxyguanosine 5′-monophosphate (dGMP); deoxyguanylate[a]
Cytosine (C)	Deoxycytidine	Deoxycytidine 5′-monophosphate (dCMP); deoxycytidylate[a]
Thymine (T)	Deoxythymidine or thymidine	Deoxythymidine 5′-monophosphate (dTMP); deoxythymidylate[a] or thymidylate[a]

[a]Anionic forms of phosphate esters predominant at pH 7.4.

Fig 19.9 Structures of the deoxyribonucleoside-5'-monophosphates



2′-Deoxyadenosine 5′-monophosphate
(Deoxyadenylate, dAMP)



2′-Deoxyguanosine 5′-monophosphate
(Deoxyguanylate, dGMP)

Draw the Following Nucleotides

- GDP
- ATP
- cGMP

Fig 19.9 (continued)

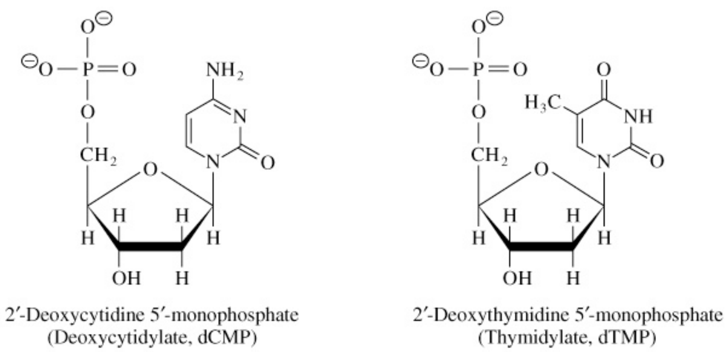
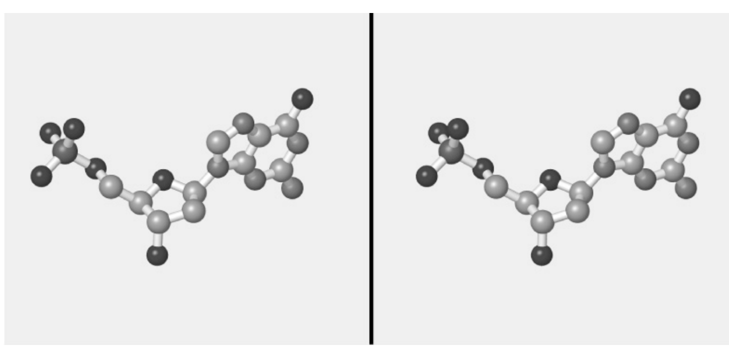


Fig 19.10 Stereo view of dGMP

- Carbon (gray), nitrogen (blue), oxygen (red), phosphorous (orange) (Hydrogens omitted)



19.2 DNA Is Double-Stranded

Table 19.2

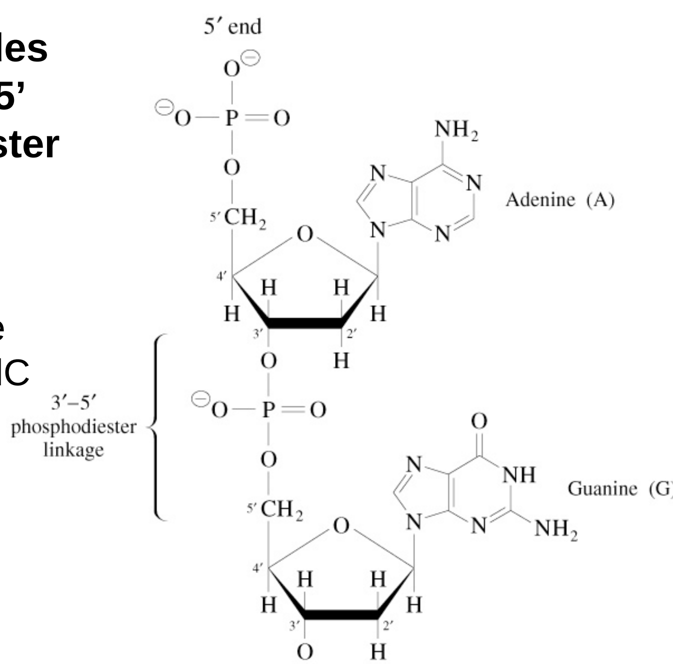
TABLE 19.2 Base composition of DNA (mole%) and ratios of bases

Source	A	G	C	T	A/T[a]	G/C[a]	(G + C)	Purine/pyrimidine[a]
Escherichia coli	26.0	24.9	25.2	23.9	1.09	0.99	50.1	1.04
Mycobacterium tuberculosis	15.1	34.9	35.4	14.6	1.03	0.99	70.3	1.00
Yeast	31.7	18.3	17.4	32.6	0.97	1.05	35.7	1.00
Cow	29.0	21.2	21.2	28.7	1.01	1.00	42.4	1.01
Pig	29.8	20.7	20.7	29.1	1.02	1.00	41.4	1.01
Human	30.4	19.9	19.9	30.1	1.01	1.00	39.8	1.01

[a]Deviations from a 1:1 ratio are due to experimental variations.

A. Nucleotides joined by 3'-5' phosphodiester linkages

Structure of the tetranucleotide pdApdGpdTpdC



B. Two Antiparallel Strands Form a Double Helix

- **Figure 19.12** (next slide)
- Two strands run in opposite directions
- Bases in opposite strands pair by <u>complementary</u> <u>hydrogen</u> <u>bonding</u>
- Adenine (A) - Thymine (T)
- Guanine (G) - Cytosine (C)

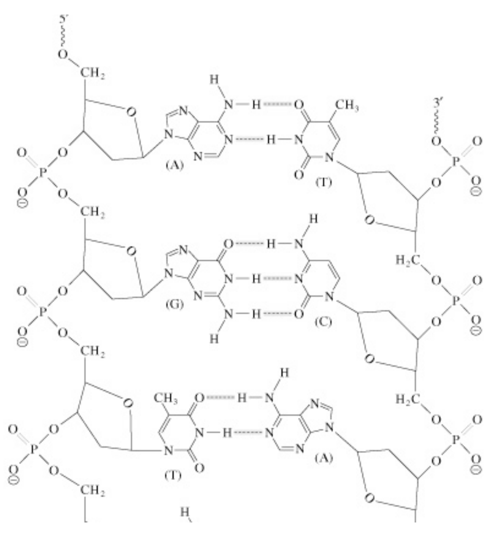
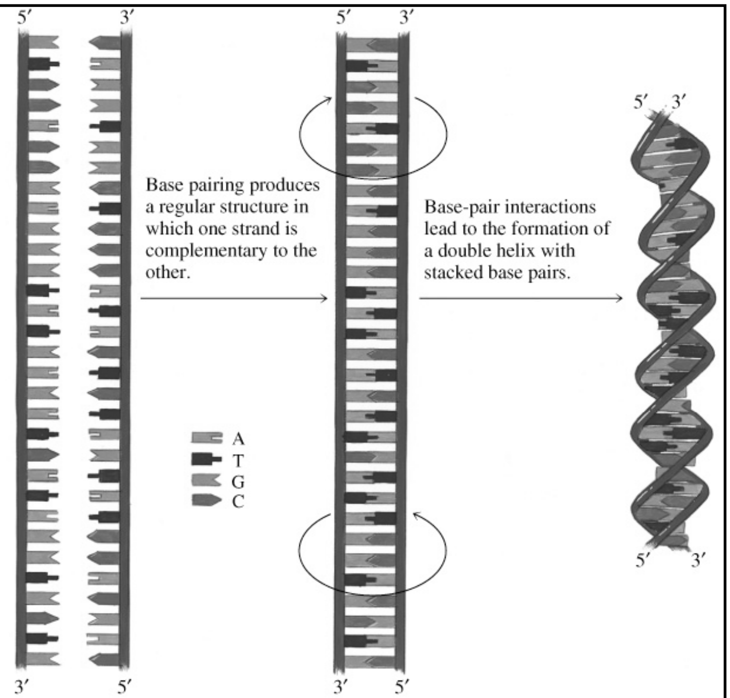


Fig 19.13

- Complementary base pairing and stacking in DNA

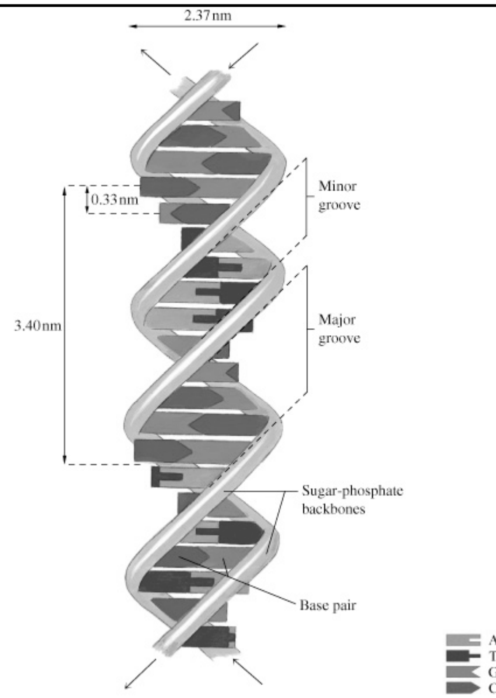


Three dimensional structure of DNA

- A double helix has two grooves of unequal width: **major groove** and **minor groove**
- Within each groove base pairs are exposed and are accessible to interactions with other molecules
- DNA-binding proteins can use these interactions to “read” a specific sequence

Fig 19.14

- Structure of B-DNA
- Sugar phosphate backbone outside
- Stacking creates two unequal grooves (major and minor)



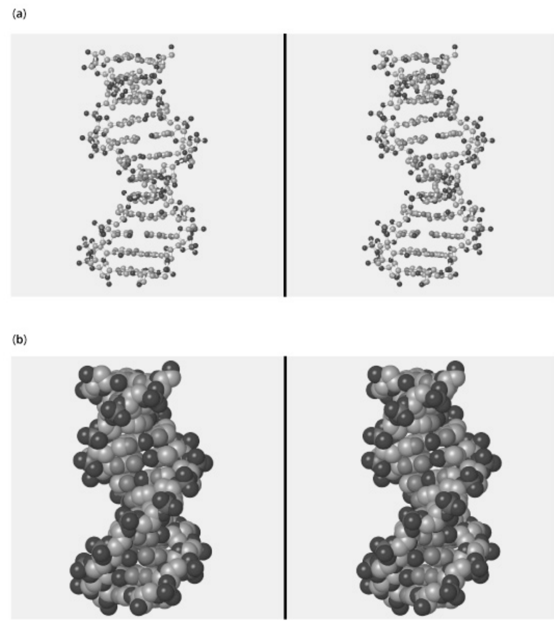
Stereo view of B-DNA

B-DNA is a right-handed helix, diam. = 2.37nm

- **Rise** (distance between stacked bases) =0.33nm
- **Pitch** (distance to complete one turn) = 3.40 nm
- Base pairs nearly perpendicular to sugar-phosphate backbones
- **Figure 19.15** Stereo views of B-DNA (next slide)

(a) Ball-and-stick model

(b) Space-filling model



C. Weak Forces Stabilize the Double Helix

(1) *Hydrophobic effects*. Burying purine and pyrimidine rings in the double helix interior

(2) *Stacking interactions*. Stacked base pairs form van der Waals contacts

(continued next slide)

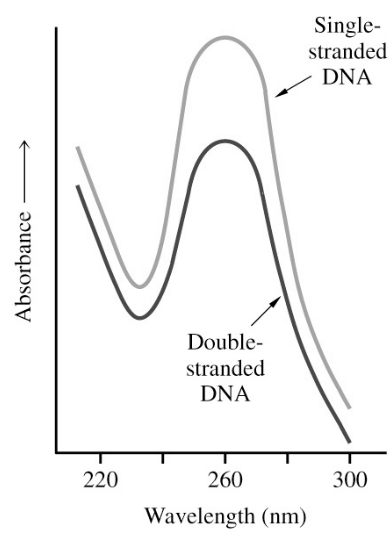
Weak forces (continued)

(3) *Hydrogen bonds*. Hydrogen bonding between base pairs.

(4) *Charge-charge interactions*. Electrostatic repulsion of negatively charged phosphate groups is decreased by cations (e.g. Mg^{2+}) and cationic proteins

Fig 19.16 Absorption spectra of double-stranded and single-stranded DNA

- Double-stranded (DS)DNA (pH 7.0), absorbance max 260nm
- Denatured DNA absorbs12% -40% more than DS DNA



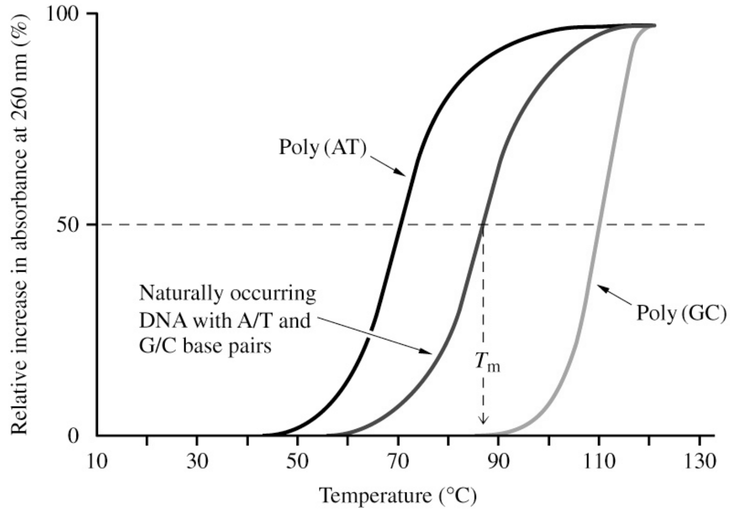
Denaturation of DNA

- Double-stranded DNA is thermodynamically more stable than the separated strands (under physiological conditions)
- **Denaturation** - Complete unwinding and separation of the 2 strands of DNA
- Heat or chaotropic agents (e.g. urea) can denature DNA

Heat denaturation of DNA

- **Melting point** (T_m) - temperature at which 1/2 of the DNA has become single stranded
- **Melting curves** can be followed at Abs_{260nm}

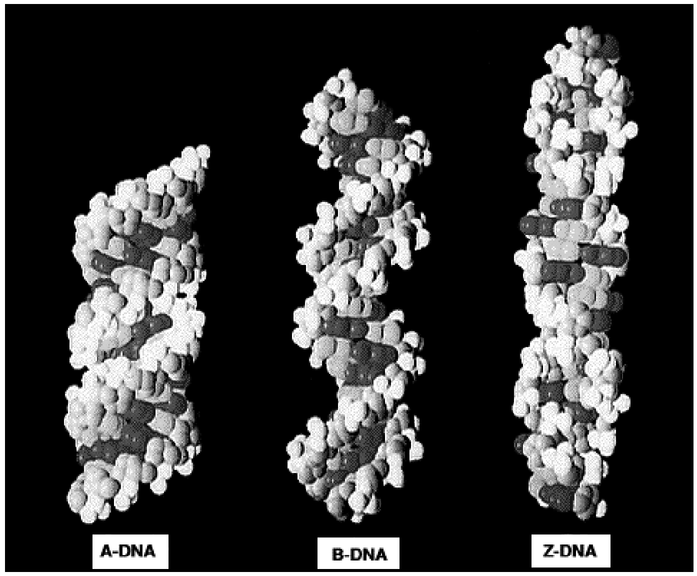
Fig 19.17 Melting curve for DNA



D. Conformations of Double-Stranded DNA

- Two alternative structures to B-DNA:
  - **A-DNA** (forms when DNA is dehydrated)
  - **Z-DNA** (when certain sequences are present)
- A-DNA is more tightly wound than B-DNA, and has minor grooves of similar width
- Z-DNA has no grooves and a left-handed helix
- Both A-DNA and Z-DNA exist in vivo in short regions of DNA

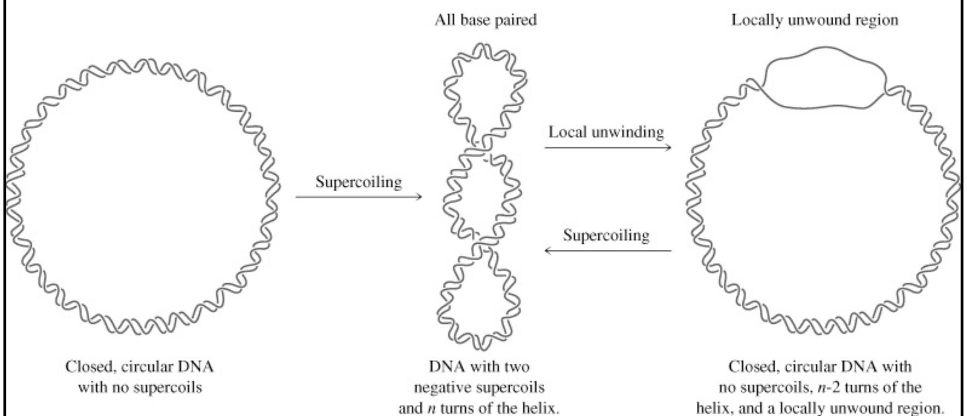
Fig 19.18 Forms of DNA

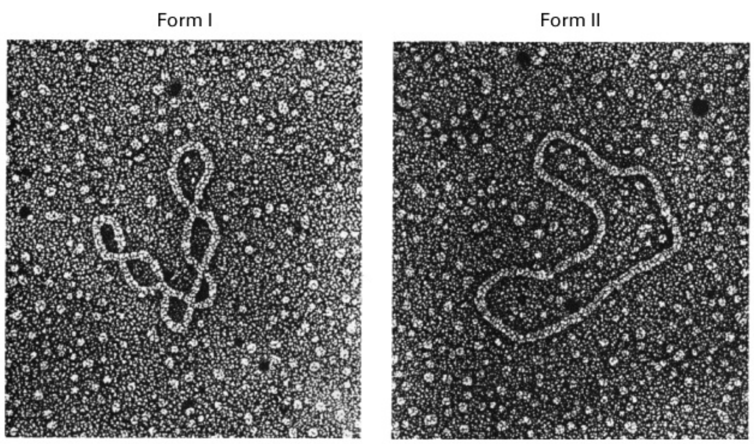


19.3 DNA Can Be Supercoiled

- “Relaxed” circular DNA with the B conformation (10.4 base pairs/turn) would lie flat on a surface
- If strands are broken, and two ends of linear DNA twisted in opposite directions and rejoined, DNA supercoils to restore 10.4 bp/turn

Fig 19.19 Structure of supercoiled DNA





supercoiled relaxed

Supercoiling

- Most bacterial chromosomes are supercoiled, and regions of eukaryotic DNA are supercoiled
- **Topoisomerases** - enzymes that can alter the topology of DNA helixes by:
  (1) Cleaving one or both DNA strands
  (2) Unwinding or overwinding the double helix by rotating the strands
  (3) Rejoining ends to create (or remove) supercoils

Fig 19.20 Human topoisomerase I bound to DNA

- Topoisomerases can add or remove supercoils in DNA
- Cleave one or both DNA strands, unwind or overwind by rotating cleaved ends, then rejoin ends

19.5 DNA Is Packaged in Chromatin in Eukaryotic Cells

- **Chromatin** - DNA plus various proteins that package the DNA in a more compact form
- The *packing ratio:* difference between the length of the metaphase DNA chromosome and the extended B form of DNA is 8000-fold

A. Nucleosomes

- **Histones** - the major proteins of chromatin
- Eukaryotes contain five small, basic histone proteins containing many lysines and arginines:
  H1, H2A, H2B, H3, and H4
- Positively charged histones bind to negatively-charged sugar-phosphates of DNA

Table 19.3

TABLE 19.3 Basic and acidic residues in mammalian histones

Type	Molecular weight	Number of residues	Number of basic residues	Number of acidic residues
Rabbit thymus H1	21 000	213	65	10
Calf thymus H2A	14 000	129	30	9
Calf thymus H2B	13 800	125	31	10
Calf thymus H3	15 300	135	33	11
Calf thymus H4	11 300	102	27	7

Nucleosomes

- **Nucleosome** “beads” are DNA-histone complexes on a “string” of double-stranded DNA
- Each nucleosome is composed of:

  Histone H1(1 molecule)

  Histones H2A, H2B, H3, H4 (2 molecules each)

  ~200 bp of DNA

The DNA strand containing genes are coiled around a protein called <u>histones</u>. The DNA and histone together are called <u>nucleosomes</u>.

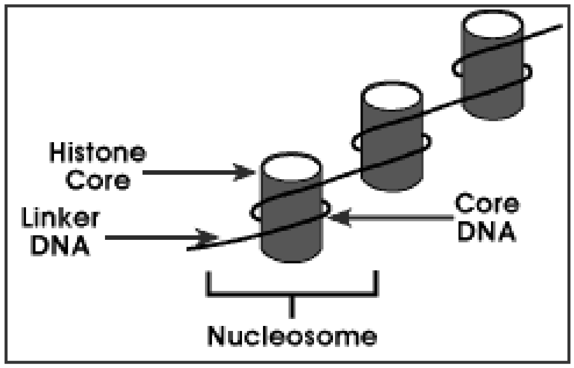


Fig 19.22 Electron micrograph of chromatin

- Chromatin "beads-on-a-string" organization

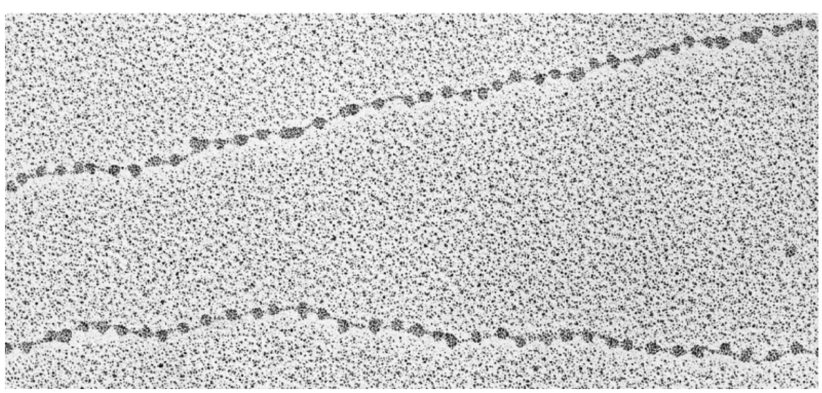


Fig 19.23 Diagram of nucleosome structure

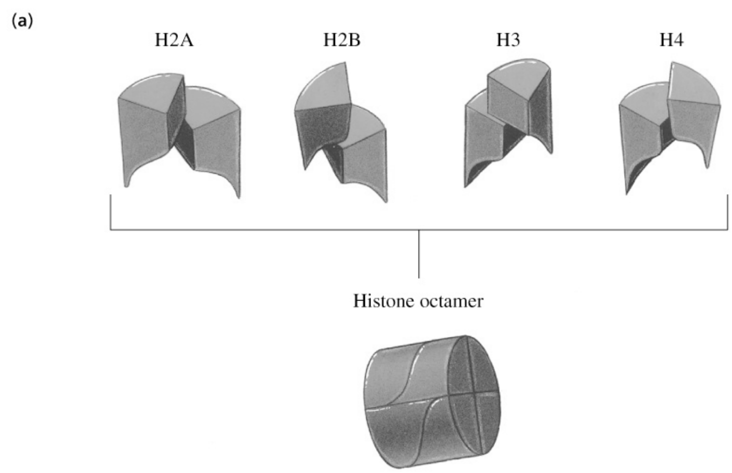


Fig. 19.23 (b)

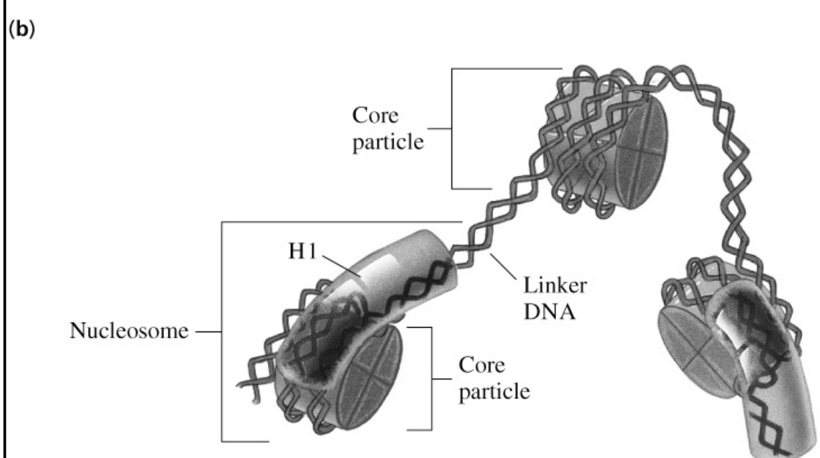
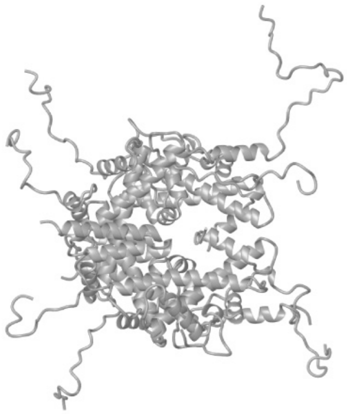


Fig 19.24 Structure of chicken nucleosome core particle

Histone octamer



Octamer bound to DNA

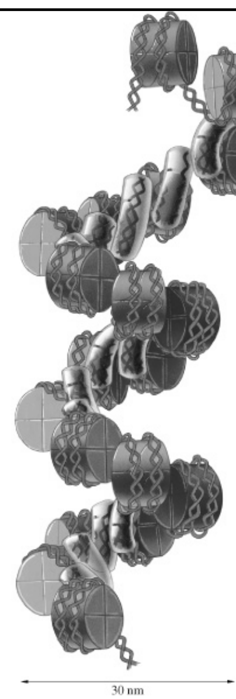
B. Higher Levels of Chromatin Structure

- Packaging of DNA in nucleosomes reduces DNA length ~tenfold
- DNA is packaged further by coiling of the "beads-on-a-string" into a solenoid structure
- Achieves another fourfold reduction in chromosome length

Solenoid model of 30nm chromatin structure

- **Figure 19.25** (next slide)
- Model of the 30nm chromatin fiber shown as a solenoid or helix formed by individual nucleosomes
- Nucleosomes associate through contacts between adjacent histone H1 molecules

Fig 19.25

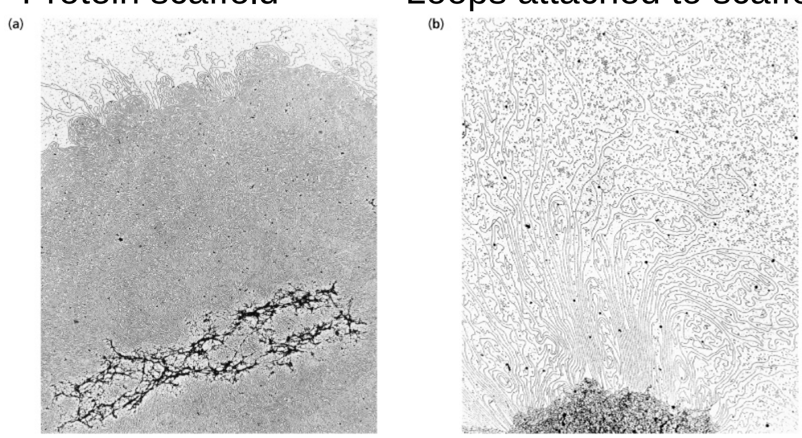


Scaffolds in chromatin

- Chromatin fibers attach to scaffolds
- Holds DNA fibers in large loops
- May be ~2000 loops on a large chromosome
- This accounts for an additional 200-fold condensation in DNA length

Fig 19.26 Histone-depleted chromosome scaffold

Protein scaffold Loops attached to scaffold



C. Bacterial DNA Packaging

- Prokaryotic DNA also packaged with proteins in a condensed form
- No defined nucleosome-like particles
- Nucleoid structure - bacterial DNA attached to a scaffold in large loops of ~100 kb

17.4 Cells Contain Several Kinds of RNA

- ***Ribosomal RNA*** (**rRNA**) - an integral part of ribosomes, accounts for ~80% of RNA in cells
- ***Transfer RNA*** (**tRNA**) - carry activated amino acids to ribosomes for polypeptide synthesis (small molecules 73-95 nucleotides long)

Types of RNA (continued)

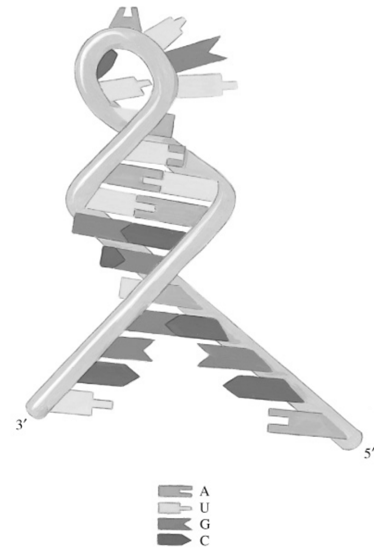
- ***Messenger RNA*** (**mRNA**) - carry sequence information to the translation complex
- ***Small RNA*** - have catalytic activity or associate with proteins to enhance activity

RNA structure

- RNA's are single-stranded molecules
- Often have complex secondary structures
- Can fold to form stable regions of base-paired, double-stranded RNA
- Example is stem-loop (hairpin) structure

Fig 19.21 Stem-loop structures in RNA

- Stem-loops or hairpins can form from short regions of complementary base pairs
- Stem: base-paired nucleotides
- Loop: noncomplementary nucleotides

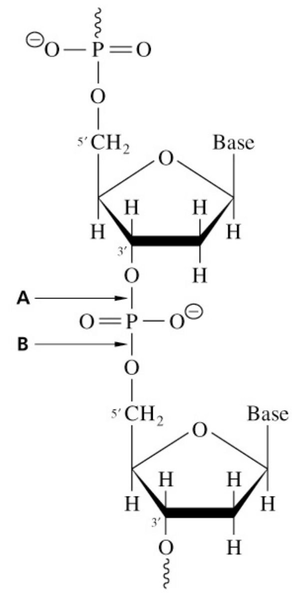


19.6 Nucleases and Hydrolysis of Nucleic Acids

- **Nucleases** - hydrolyze phosphodiester bonds
  RNases (RNA substrates)
  DNases (DNA substrates)
- May cleave either the 3’- or the 5’- ester bond of a 3’-5’ phosphodiester linkage
- **Exonucleases** start at the end of a chain
- **Endonucleases** hydrolyze sites within a chain

Fig 19.27

- Nuclease cleavage sites
- Cleavage at bond A generates a 5'-phosphate and a 3' OH terminus
- Cleavage at bond B generates a 3'-phosphate and a 5'-hydroxyl terminus



A. Alkaline Hydrolysis of RNA

Fig 19.28

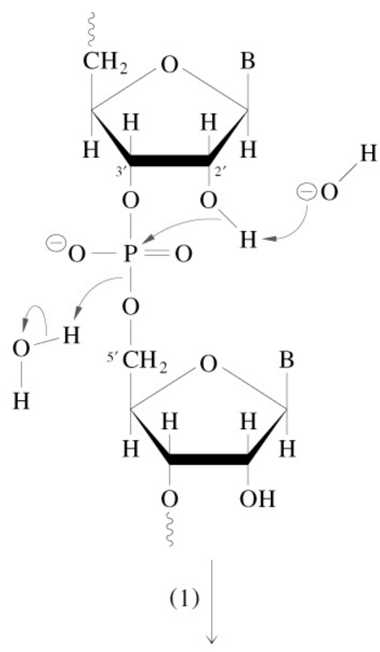


Fig 19.28 (cont)

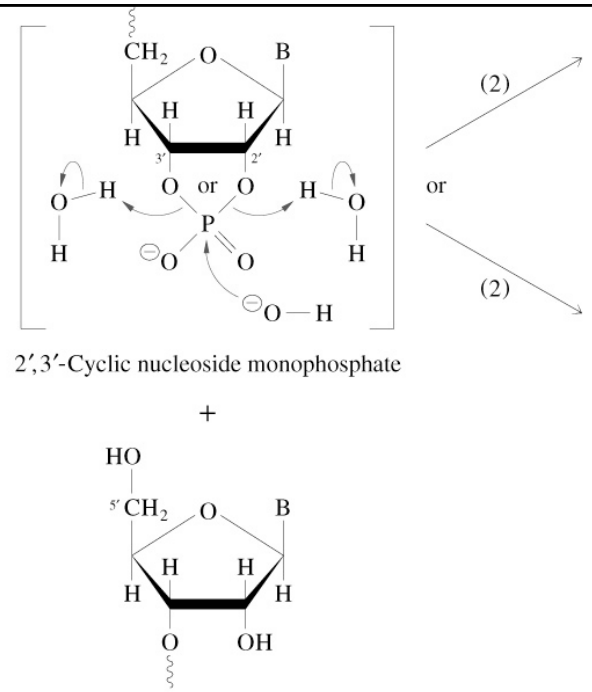
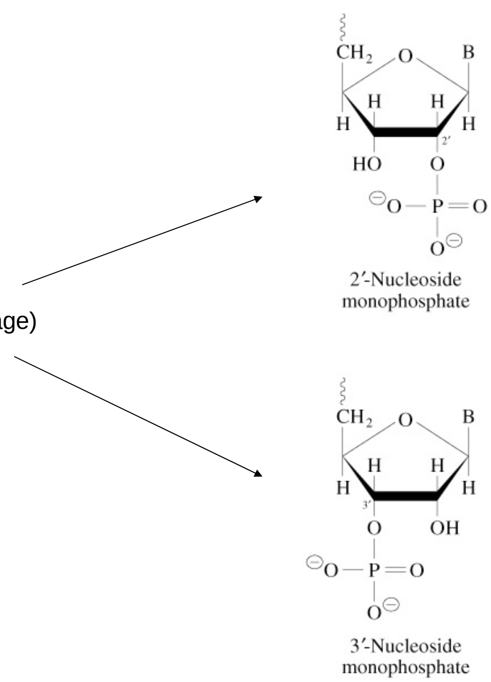


Fig 19.28 (cont)

(From previous page)



B. Ribonuclease-Catalyzed Hydrolysis of RNA

Fig 19.29

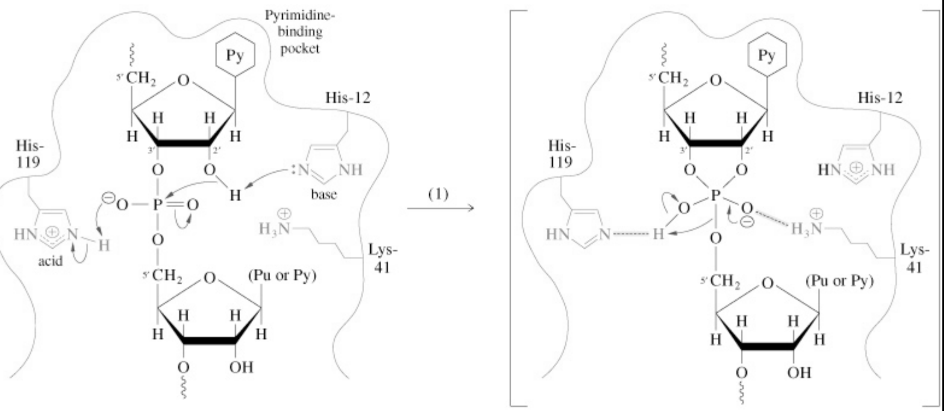


Fig 19.29 (cont)

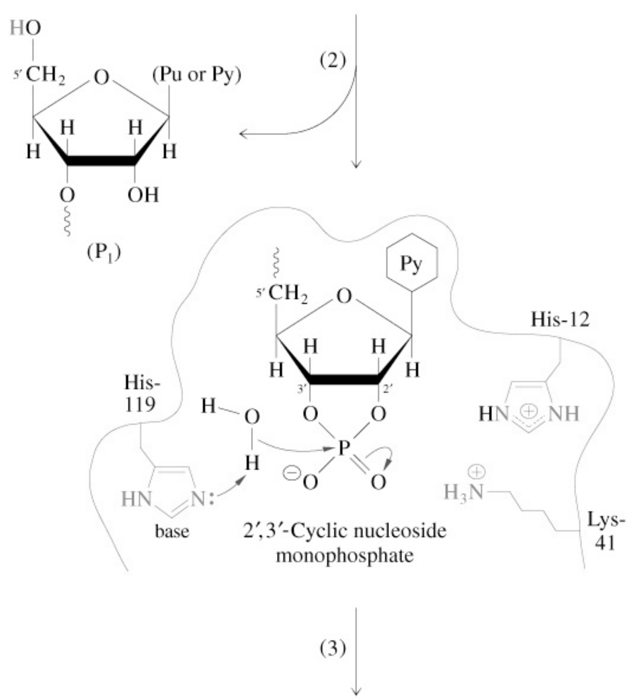
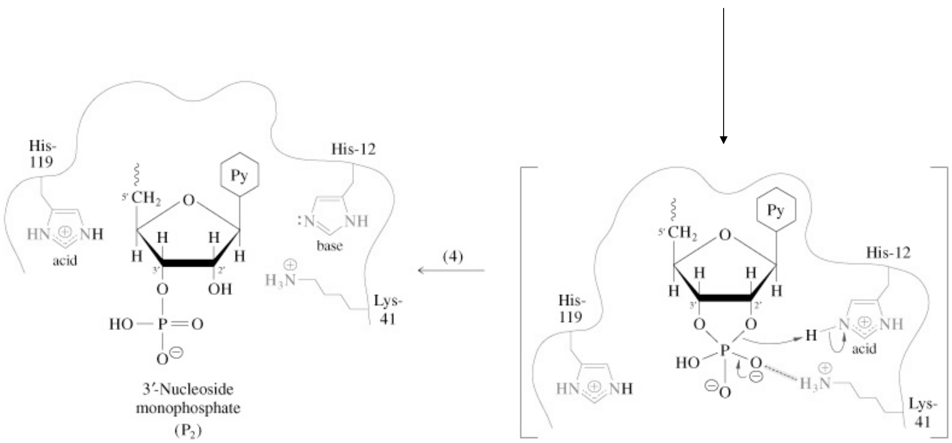


Fig 19.29 (cont)



C. Restriction Endonucleases

- Enzymes that recognize specific DNA sequences
- Cut both strands of DNA at the binding site, producing fragments that can be degraded by exonucleases
- Host cells protect their own DNA by covalent modification of bases at the restriction site (e.g. methylation)

Restriction endonuclease properties

- **Type I** - catalyze both the methylation of host DNA and cleavage of unmethylated DNA at a specific recognition sequence
- **Type II** - cleave double-stranded DNA only, at or near an unmethylated recognition sequence
- More than 200 type I and type II are known
- Most recognize “palindromic sequences” (read the same in either direction)

TABLE 19.4 Specificities of some common restriction endonucleases

Source	Enzyme[a]	Recognition sequence[b]
Acetobacter pasteurianus	*Apa* I	GGGCC↓C
Bacillus amyloliquefaciens H	*Bam* HI	G↓GATCC
Escherichia coli R Y13	*Eco* RI	G↓AA*TTC
Escherichia coli R245	*Eco* RII	↓CC*TGG
Haemophilus aegyptius	*Hae* III	GG↓CC
Haemophilus influenzae R_d	*Hin* dIII	A*↓AGCTT
Haemophilus parainfluenzae	*Hpa* II	C↓CGG
Klebsiella pneumoniae	*Kpn* I	GGTAC↓C
Nocardia otitidis-caviarum	*Not* I	GC↓GGCCGC
Providencia stuartii 164	*Pst* I	CTGCA↓G
Serratia marcescens S_b	*Sma* I	CCC↓GGG
Xanthomonas badrii	*Xba* I	T↓CTAGA
Xanthomonas holcicola	*Xho* I	C↓ G

Fig 19.30

- Methylation and restriction at the *Eco*R1 site

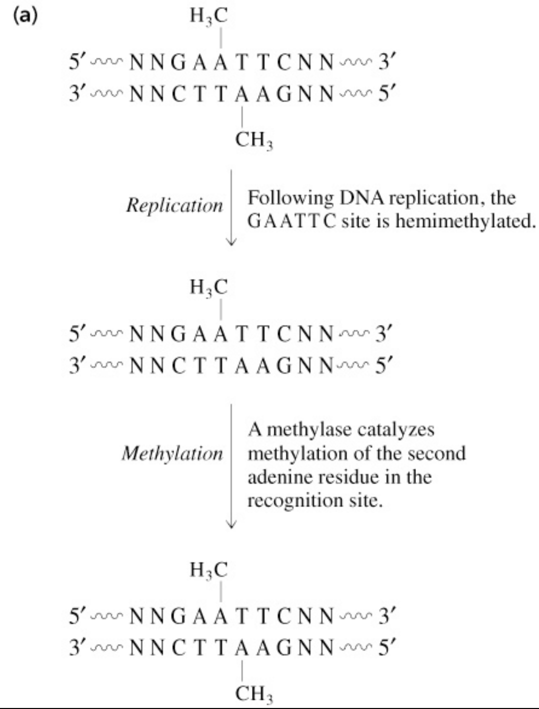
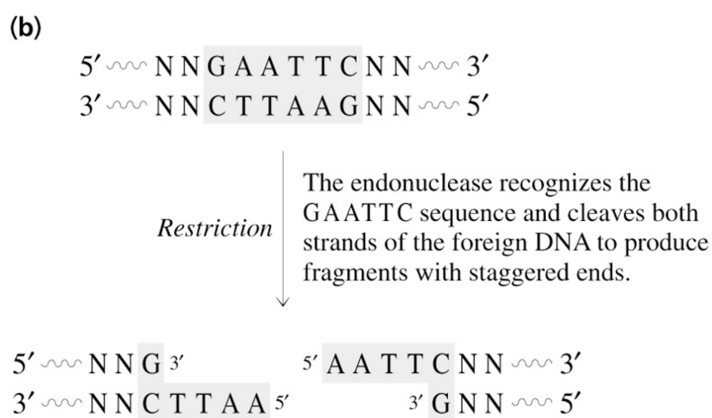
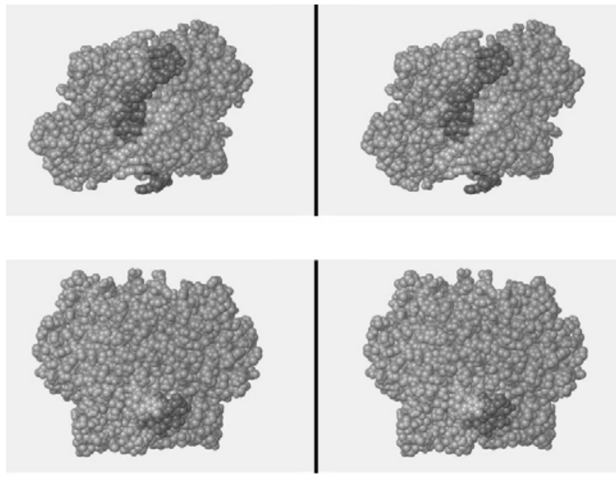


Fig 19.30 (continued)



D. *Eco*R1 Binds Tightly to DNA

- *Eco*R1 has 2 identical subunits (purple and yellow)
- Bound to a fragment of DNA (strands blue and green)



19.7 Uses of Restriction Endonucleases

- Developing restriction maps (indicates specific cleavage sites in a DNA fragment)
- Map of bacteriophage λ showing cleavage sites of some restriction enzymes

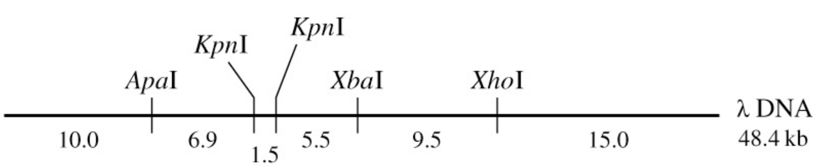
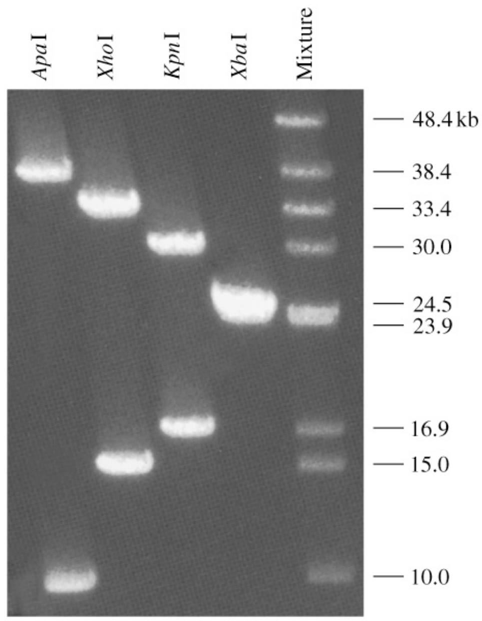


Fig 19.33

- Restriction digest of bacteriophage λ
- Four restriction enzymes used
- Sizing gel separates fragments (smallest move fastest)



DNA Fingerprinting

- DNA sequence can be used to identify individuals in a large population
- Highly variable regions give restriction fragments that are as unique as fingerprints

Fig 19.34

- DNA Fingerprinting